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Supplemental Data

Rapid E2-E3 Assembly and Disassembly

Enable Processive Ubiquitylation of

Cullin-RING Ubiquitin Ligase Substrates

Gary Kleiger, Anjanabha Saha, Steven Lewis, Brian Kuhlman, and Raymond J. Deshaies

Docking of the acidic tail to the Cul1 and Cul5 CTD

Generation of starting models

No crystal structure exists of the Cul1-Rbx1-Cdc34 complex, with or without Nedd8 attached. Additionally, no structure exists of the flexible Cdc34 tail we were interested in modeling. To create the requisite starting models, we used a combination of structural alignments to homologous structures and computational docking.

Cul1-Rbx1-Cdc34 was pieced together from PDB files 2OB4 (human Cdc34) and 1LDJ (Cul1-Rbx1) (Zheng et al., 2002). Cul1 was trimmed to residues 411-776. To approximate the E2-RING interaction, these two PDB files were aligned against the E2-RING complex CBL-UBCH7, 1FBV (Zheng et al., 2000). This docking alignment was then refined using Rosetta's docking tools (Gray et al., 2003). A similar model complex was generated in the same fashion from PDB files 2OB4 (Cdc34) and 3DQV (chains A, C, and R) (Cul5-Rbx1-Nedd8) (Duda et al., 2008).

The final step of generating starting models was attaching the tail sequence to Cdc34 (not seen in the crystal structure). We used the simple expedient of extending the tail straight out into space from the C-terminal residue in the Cdc34 crystal structure.

Modeling the flexible tail

To explore how the flexible tail might interact with the E3 in this system, we built a new protocol within the Rosetta framework. Figure (S8) demonstrates

the flow of this protocol. The protocol has a reduced-representation “centroid” phase and a second refinement phase with all atoms present.

The centroid phase is designed to collapse the long tail, which initially points straight into space, into some reasonable conformation. The reduced representation has only single large “centroid” atoms replacing the side-chains (Rohl et al., 2004), obviating the need to consider packing details. The advantage of using this representation in the early part of the trajectory is that we no longer need to repack side-chains to smooth out minor side-chain/side-chain clashes, thus speeding up the computation. This phase consisted of 5,000 Metropolis Monte Carlo cycles. Most cycles were used to perturb the tail structure with one of three Rosetta tools (Rohl et al., 2004).

The first tool, marked as Small_180 on Figure S8, randomly perturbed a phi and psi angle of a tail residue, weighted to choose good Ramachandran values. The second, Shear_180 on Figure S8, modifies a phi angle at position n and psi at position $n-1$ in opposite directions. This has the effect of tweaking local structure without longer-distance effect. Again, this was a random perturbation subject to Ramachandran constraints. Shear movements are designed to minimize perturbation of the backbone far from the move, so they were disabled for the first third of the protocol to give the tail a chance to develop interactions before their use.

The third type of perturbation was a 3-residue fragment insertion. Fragment insertion consists of replacing the backbone torsional parameters with parameters from a small protein fragment derived from the PDB. Fragments were generated via the Robetta server (Kim et al., 2004). This allowed for somewhat faster sampling of local conformational and secondary structure preferences.

After 19 cycles, the protocol performed a gradient-based energy minimization of the structure. The available degrees of freedom were the backbone torsion angles phi, psi and omega of tail residues (the same freedoms perturbed in the other steps). The score function used in this phase contained terms for van der Waals repulsion, hydrogen bonding, electrostatics,

Ramachandran, and residue-pair potentials (which incorporate van der Waals attraction and solvation) (Rohl et al., 2004).

After 5,000 perturbing cycles, the lowest-scoring structure seen in the trajectory was recovered. Side-chains were returned to this structure. The crystallographic side-chains were used outside the tail. One of Rosetta's default full atomic representation energy functions, score12, was used for scoring in this phase (Kuhlman et al., 2003) (Smith and Kortemme, 2008). The tail region and any side-chains within 10 Ångstroms of the tail were subjected to a combinatorial repacking wherein a Monte Carlo procedure chose low-energy side-chain conformations from the full set of available rotamers. The side-chains and the tail backbone torsions were then energy minimized. The refinement phase ran for 3,000 cycles of Metropolis Monte Carlo optimization. Most cycles consisted of a random small perturbation. These perturbations were small or shear movements as before, except the perturbation was constrained to within 4 degrees, instead of anywhere in Ramachandran space. This sort of perturbation was immediately followed by a single-pass random-order rotamer optimization of the tail and its neighbors (rotamer trials). After 14 cycles, a minimization of the tail side-chains, tail backbone torsions, and side-chains neighboring the tail was performed. Another 14 cycles after that, a full combinatorial repacking operation was performed before the minimization. At the end of each trajectory, the best structure recorded was reported as output.

The protocol as a whole was run for approximately 30,000 trajectories per experiment, which is slightly more than 1,000 processor-days on a 2.3 GHz chip. It is customary to run extremely large numbers of trajectories when doing structure prediction with Rosetta. The random nature of Monte Carlo sampling means that trajectories will sometimes get trapped in poor minima, so many trajectories are needed to sufficiently sample the structure space.

Sorting models

To search through our dataset for models that addressed the question of how the E2 might bind the E3, we sorted models based on E2-E3 binding energy. This was calculated as the complex score minus the scores of the E2

and E3-RING (\pm Nedd8) in isolation. It should be noted that there are two components to this binding energy. There is a constant component represented by the direct, canonical E2-RING interaction. This interaction was optimized by docking as a precursor step but not modified during the main part of the protocol. The second interaction represents binding between the E2 tail and the body of the E3.

Supplemental References

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Supplemental Figure Legends

Figure S1. Multiple Sequence Alignment of the human Cdc34a, human Cdc34b, and yeast Cdc34 1 - 230 sequences. While the yeast Cdc34 sequence is 295 amino acids, 1 – 230 was included during the alignment procedure (MUSCLE) and resulted in a better alignment than when the full-length sequence was used. Amino acids within the catalytic domain are shaded olive. Amino acids within the acidic tail domain are shaded light-blue. Acidic residues in the tail domain are shaded yellow.

Figure S2. The effects of Cul1 neddylation and ubiquitin oxyesterification to Cdc34 on k_{on} are less than 2-fold. All experiments were performed identically to those in Figure 1a. (A) Measurement of k_{off} for Lumio Green labeled WT Cdc34 and neddylated RC^{CFP} complex. k_{on} was estimated from k_{off} and K_d was taken from (Saha and Deshaies, 2008). (B) Measurement of k_{off} for WT Cdc34 that was oxyestified to ubiquitin and neddylated RC^{CFP} complex. k_{on} was estimated from k_{off} and K_d was taken from (Saha and Deshaies, 2008). (C) Measurement of k_{off} for WT Cdc34 and a full SCF ^{β TrCP} complex. Equimolar β TrCP-Skp1 was added to the Lumio Cdc34-RC^{CFP} complex. Notice that the value of k_{off} is very nearly the same whether the full SCF complex is assembled or not (27 and 35 sec⁻¹, respectively). The ionic strength of the reaction buffer for all experiments was 50 mM.

Figure S3. Binding assays to RC^{CFP} with N-terminally labeled Lumio Green Cdc34 protein show similar results as when the Lumio tag is at the C-terminus. (A) Salt sensitivity of FRET efficiency of RC^{CFP} complex saturated with C-terminally labeled WT Cdc34 (white bars) or RC^{CFP} complex with nearly saturating N-terminally labeled WT Cdc34 (black bars). Notice that N-terminally labeled Cdc34-RC^{CFP} complex is highly salt sensitive, similar to C-terminally labeled Cdc34. (B) Salt sensitivity of Δ 221 Cdc34. (C) Titration of N-terminal Lumio-labeled Cdc34 on RC^{CFP}. All experiments were done in duplicate.

Figure S4. (A) Model showing the interaction between the acidic tail and the Cul5 CTD(Nedd8)-Rbx1 structure. Cul5, Rbx1 and Nedd8 are colored according to the electrostatic potential of the surface. The location of Nedd8 in the structure is shown by the dashed gray oval. The catalytic domain of Cdc34 is shown as a yellow ribbon diagram. Cdc34 residues in the tail are yellow ball-and-sticks, except acidic residues are colored red. (B) Magnified view of (A).

Figure S5. Neddylation of KKR SCF results in a similar degree of activation as WT SCF. (A) Time course of the neddylation of WT or KKR RC. Notice that at completion the same amount of product is observed for either WT or KKR RC. (B,C) Ubiquitylation of a mono-ubiquitylated β -Catenin peptide by either WT (B) or KKR (C) SCF and 0.5 μ M WT Cdc34. Neddylation stimulated the rate of substrate consumption under multi-turnover conditions by approximately 2-fold for both WT and KKR SCF.

Figure S6. Multiple sequence alignment of 9 cullin sequences by MUSCLE. Coloring of conserved residues was performed according to definitions in ClustalX (Larkin et al., 2007). Black asterisks were placed beneath positions in the MSA corresponding to human Cul1 KR claw residues 431, 432, 435, 678, 679 and 681.

Figure S7. Cdc34- Δ 190 can be fully thioesterified to ubiquitin at concentrations as high as 100 μ M. SDS-PAGE in the absence of reducing reagent for reactions containing human E1, various concentrations of human Cdc34- Δ 190 (shown), and ubiquitin. The location of apo-E2 and the thioesterified E2-ubiquitin complex are shown. Notice that concentrations as high as 100 μ M E2 can be achieved where little or no apo-E2 is present.

Figure S8. Flow diagram illustrating the procedure used for modeling the Cdc34 acidic tail interaction with either Cul1 or neddylated Cul5 CTD.

Table S1. Constructs, expression, and purification for proteins used in this manuscript.

Protein	Construct	Strain	Expression	Purification	Comments
WT hCdc34 ^a	pET11b-hCdc34-His6	RDB2314	E.coli	NiNTA ^b ->S75 ^c	
Δ190 hCdc34	pET11b-Δ190 hCdc34-His6	RDB2315	E.coli	NiNTA->S75	
NLum ^d -WT hCdc34	pET11b-Lum-hCdc34-His6	RDB2316	E.coli	niNTA->S75	
CLum-WT hCdc34	pET11b-hCdc34-Lum-His6	RDB2317	E.coli	niNTA->S75	
CLum-Δ221 hCdc34	pET11b-Δ221 hCdc34-Lum-His6	RDB2318	E.coli	niNTA->S75	
C227S hCdc34	pET11b-C227S-hCdc34-His6	RDB2319	E.coli	niNTA->S75	
UbcH5c	pGex-TEV-UbcH5c	RDB1973	E.coli	GST->TEV digest (on beads)->S75	
Cul1 NTD Rbx1-Cul1 CTD	pAL Cul1 NTD, pCool Rbx1 Cul1 CTD	RDB2080 RDB2081	E.coli	GST-Thrombin-Mono S->S200 ^e	Co-expression
Cul1 NTD Rbx1-Cul1 CTD (CFP)	pAL Cul1 NTD, pCool Rbx1 Cul1 CTD (CFP)	RDB2080 RDB2228	E.coli	GST-Thrombin-Mono S->S200	Co-expression
Cul1 NTD Rbx1-Cul1 CTD K679C	pAL Cul1 NTD, pCool Rbx1 Cul1 CTD K679C	RDB2320	E.coli	GST-Thrombin-Mono S->S200	Co-expression
Cul1 NTD Rbx1-Cul1 CTD KKR	pAL Cul1 NTD, pCool Rbx1 Cul1 CTD KKR	RDB2321	E.coli	GST-Thrombin-Mono S->S200	Co-expression
Δ230 yCdc34 ^f	pET11b-Δ230 yCdc34-His6	RDB2322	E.coli	Ni-NTA->S75	
Δ210 yCdc34	pET11b-Δ210 yCdc34-His6	RDB2323	E.coli	Ni-NTA->S75	
βTrCP-Skp1 (139-569)	GST βTrCP-Skp1	-	Hi5 insect cell	GST->Thrombin-Mono Q->S200	Co-expression
ySCF (Hrt1, Cdc53, Skp1, Cdc4)		-	Hi5 insect cell	Py beads	Co-expression

^a human, ^b Nickel agarose purification, ^c Superdex 75 gel filtration column

^d CCPGCC Lumio tag sequence, ^e Superdex 200 gel filtration column, ^f yeast

Table S2. Reaction conditions for ubiquitylation assays in this manuscript

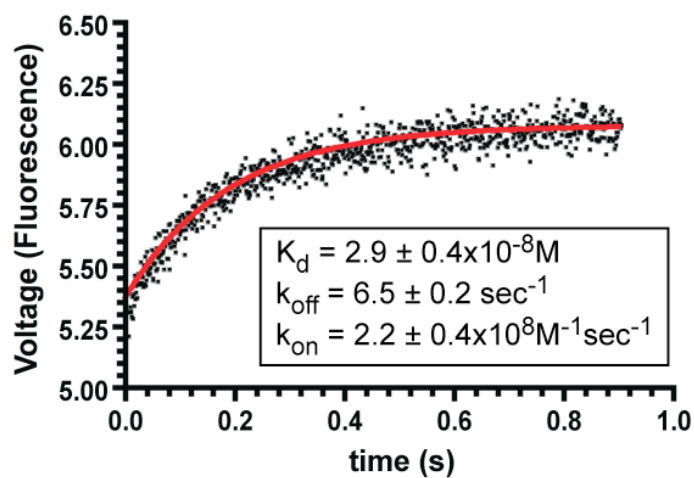
Figure	E1	Ub	E2	SCF	Substrate	Competitor
2a	hE1, 1 μ M	60 μ M	WT Cdc34 (titration series)	WT Cul1-N8, Rbx1, Skp1, β TrCP (0.15 μ M)	β Catenin ^{Ub} peptide (4 μ M)	
2b	hE1, 1 μ M	150 μ M	Δ 190 Cdc34 (titration series)	WT Cul1-N8, Rbx1, Skp1, β TrCP (0.15 μ M)	β Catenin ^{Ub} peptide (4 μ M)	
5a	hE1, 1 μ M	60 μ M	WT Cdc34 (titration series)	KKR Cul1, Rbx1, Skp1, β TrCP (0.1 μ M)	β Catenin ^{Ub} peptide (3 μ M)	
5b	hE1, 1 μ M	60 μ M	Δ 190 Cdc34 (9 μ M)	WT Cul1, Rbx1, Skp1, β TrCP (0.4 μ M)	β Catenin ^{Ub} peptide (3 μ M)	
5b	hE1, 1 μ M	60 μ M	Δ 190 Cdc34 (9 μ M)	KKR Cul1, Rbx1, Skp1, β TrCP (0.4 μ M)	β Catenin ^{Ub} peptide (3 μ M)	
5c	hE1, 1 μ M	60 μ M	Ubc5c (0.75 μ M)	WT Cul1, Rbx1, Skp1, β TrCP (0.2 μ M)	β Catenin peptide (5 μ M)	
5c	hE1, 1 μ M	60 μ M	Ubc5c (0.75 μ M)	KKR Cul1, Rbx1, Skp1, β TrCP (0.2 μ M)	β Catenin peptide (5 μ M)	
5d	yE1, 1 μ M	300 μ M	Δ 230 yCdc34 (10 μ M)	ySCF (0.35 μ M)	Cyclin E peptide (30 nM)	Cyclin E peptide (20 μ M)
5d	yE1, 1 μ M	300 μ M	Δ 210 yCdc34 (10 or 100 μ M)	ySCF (0.35 μ M)	Cyclin E peptide (30 nM)	Cyclin E peptide (20 μ M)

Figure S1

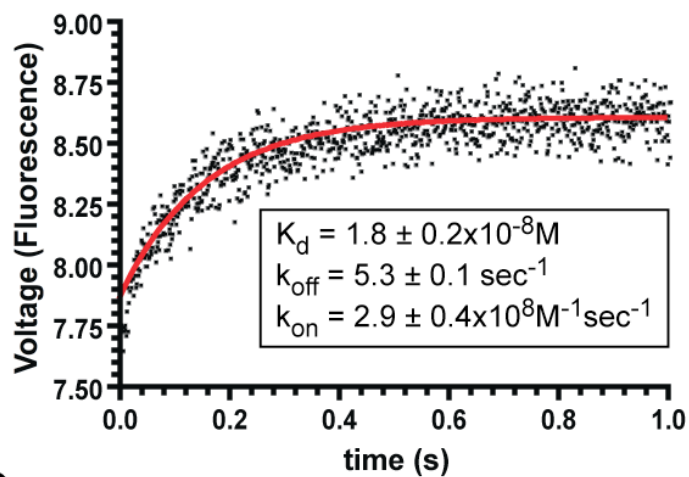
<i>cdc34 Yeast /1-230</i>	1	-MSSRKSTASSLLLRQYRELTPKKAIPSFHIELED	35
<i>ubc3-a 1-236</i>	1	MARPLVPSSQKALLLELKGLQE--EPVEGFRVT	34
<i>UBC3B HomoSapiens /1-238</i>	1	MAQQQMTSSQKALMLELKSLE--EPVEGFRIT	34
<i>cdc34 Yeast /1-230</i>	36	DSNIFTWNIGVMVLNEDSIYHGGFFKAQMRFPEDFP	71
<i>ubc3-a 1-236</i>	35	EGDLYNWEVAIFG-PPNTYYEGGYFKARLKFPIDYP	69
<i>UBC3B HomoSapiens /1-238</i>	35	ESDLYNWEVAIFG-PPNTLYEGGYFKAHIKFPIDYP	69
<i>cdc34 Yeast /1-230</i>	72	FSPPPQFRFTPAIYHPNVYRDGRLCISILHQS-GDPM	106
<i>ubc3-a 1-236</i>	70	YSPPAFRFLT KMWHPNIYETGDVCISILHPPVDDPQ	105
<i>UBC3B HomoSapiens /1-238</i>	70	YSPPTFRFLT KMWHPNIYENG DVCISILHPPVDDPQ	105
<i>cdc34 Yeast /1-230</i>	107	TDEPDAETWSPVQTVESVLI SIVS LLEDPNINS PAN	142
<i>ubc3-a 1-236</i>	106	SGELPSERWNPTQNVRTILLSVISLLNEPNTFSPAN	141
<i>UBC3B HomoSapiens /1-238</i>	106	SGELPSERWNPTQNVRTILLSVISLLNEPNTFSPAN	141
<i>cdc34 Yeast /1-230</i>	143	VDAADVYRK-----NPEQYKQRVKMEVERSKQDIP	172
<i>ubc3-a 1-236</i>	142	VDASVMYRKWKESKGKDREYTDIIRKQVLGTVDAE	177
<i>UBC3B HomoSapiens /1-238</i>	142	VDASVMFRKWRDSKGKDKEYAEIIRKQVSATKAEAE	177
<i>cdc34 Yeast /1-230</i>	173	K-GFIMPTS ESAYISQSKLDEPESNKDMADNFWYDS	207
<i>ubc3-a 1-236</i>	178	RDGVKVPTTLAEYCVKTKAPAPDEGSDLFYDDYYED	213
<i>UBC3B HomoSapiens /1-238</i>	178	KDGVKVPTTLAEYCIKTKVPSNDNSSDLLYDDL YDD	213
<i>cdc34 Yeast /1-230</i>	208	DLDD--DENGSVILQDDDYDDGNNH-	230
<i>ubc3-a 1-236</i>	214	G-EV---EEEADSCFGDDEDDSGTEES	236
<i>UBC3B HomoSapiens /1-238</i>	214	DI DDEDEEEEDADCY--DDDDSGNEES	238

Figure S2

A



B



C

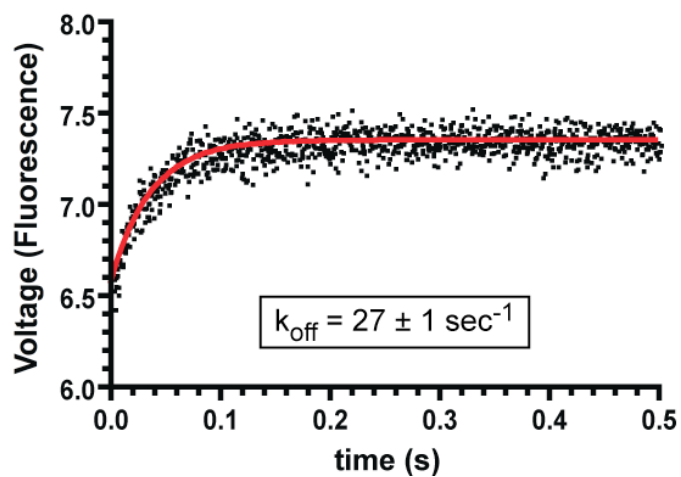
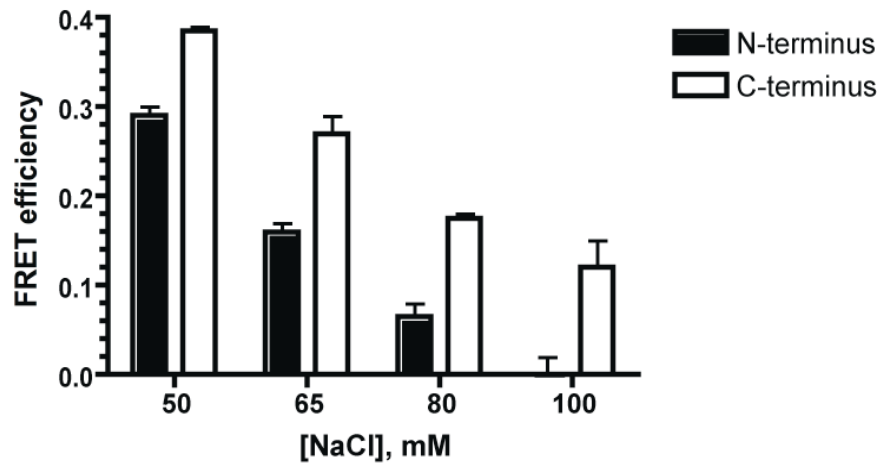
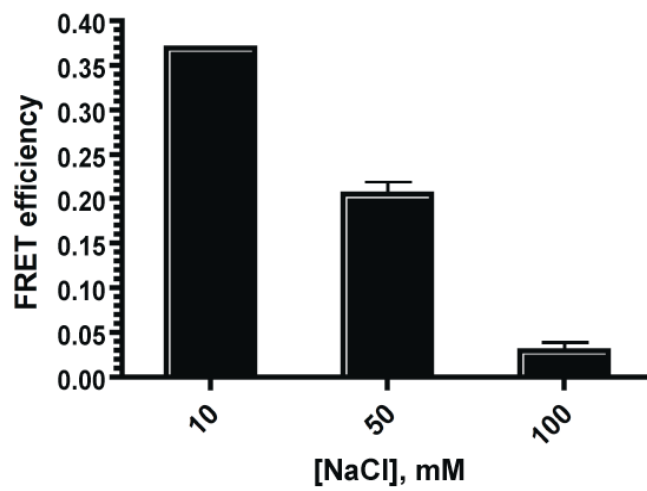


Figure S3

A



B



C

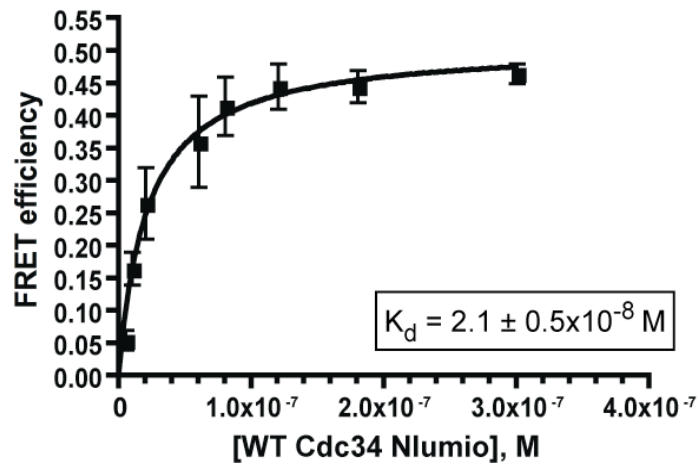
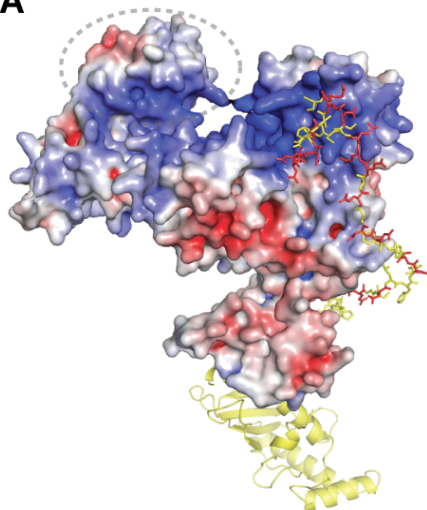


Figure S4

A



B

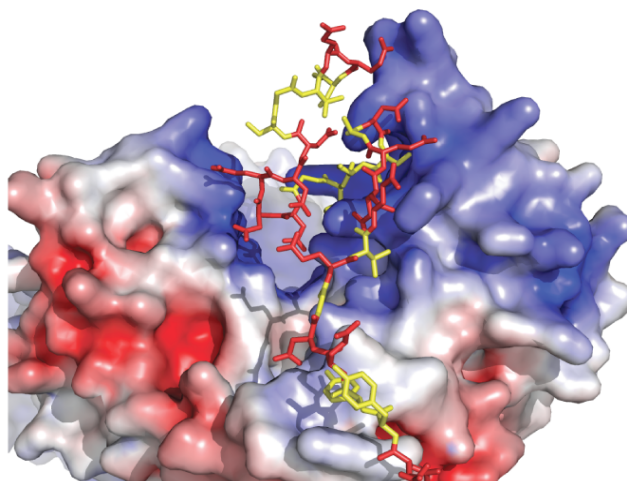


Figure S5

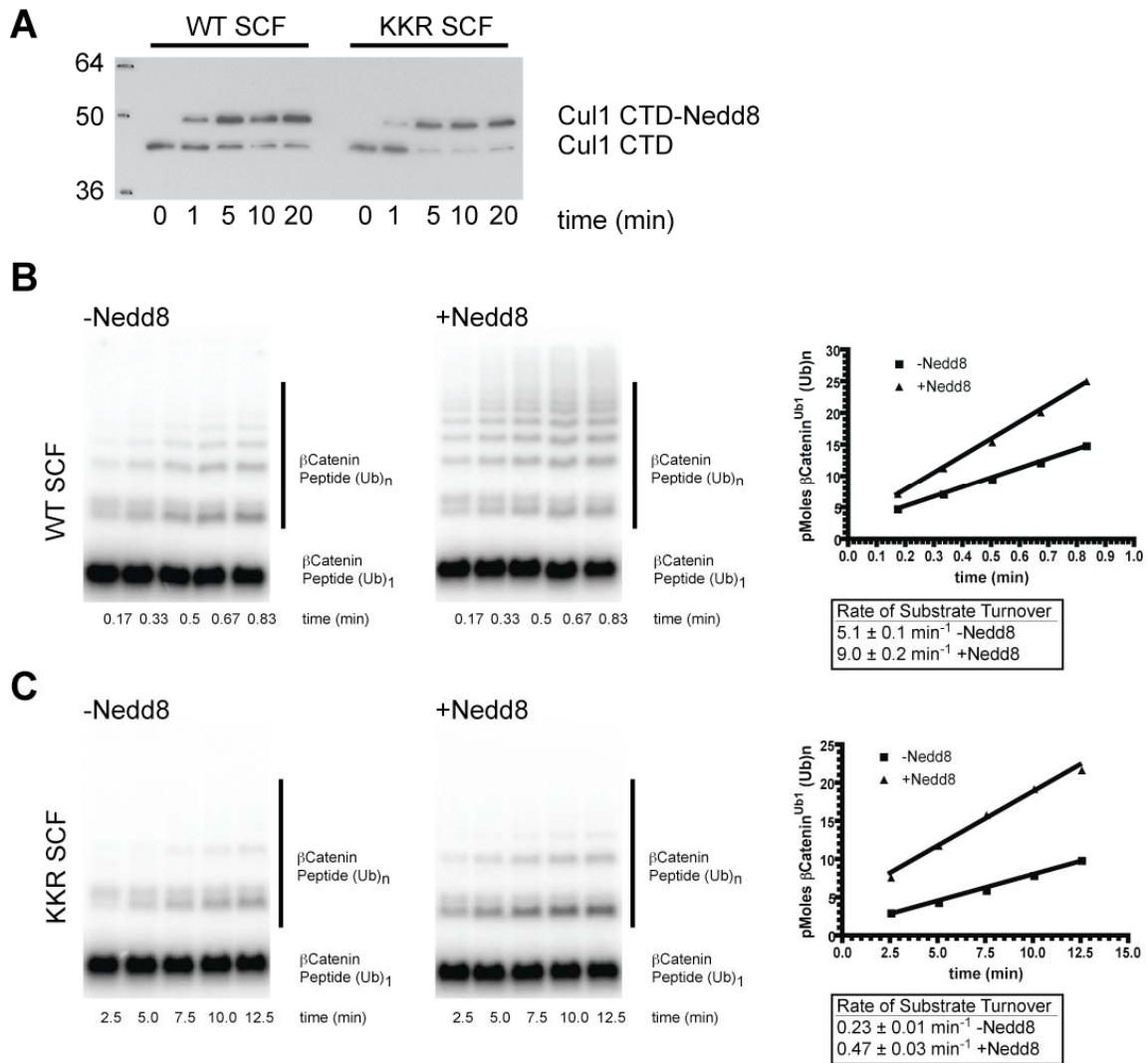


Figure S6

<i>D.melanogaster</i>	Cul1	398	ACCKFINSNV-VT-IANSA-----SKSPPELLAKYCDLLLKKS--SKNPEDKELEDNLNQVMVVKYIEDKDVFFQY	464
<i>C.elegans</i>	Cul1	399	AATSFINNNA-VTKRAPPPQAQL-----TKSAELLARYCDQLLRKS--SKMPDEAELEELQTKIMVVFYIDDKDVFSKF	469
<i>S.pombe</i>	Cul1	395	AFRELVNRRV-VCQRSS-----SRSPPELLAKYADSLRKS--NKNVDIDVVEDCLSSIIIFRYVEDKDVFFQNF	460
<i>S.cerevisiae</i>	Cul1	411	ACGAFININEFALPAGSPK SAT-----SKTSEM LAKYSDI L LKKA--TKPEVASDMSD--EDIITIFKYLTDKDAFETH	480
<i>H.sapien</i>	Cul5	372	AYKAVVNDAT-IFKLELP LKQKGVGLKTQPE--SKCPPELLANYCDMLLRKTPLSKKLTSEEIAKLEVLVLVLYVQNKDVFMRY	453
	Cul4B	547	AFETFINKRP-----NKP AELI AKYVD SKLRAC--NKEATDEELEKMLDKIMIFRFIYGGKDVFEAF	606
	Cul3	373	DFEYFLNLLN-----SRSPPEYLSLFIDDKLKKG--VKGLETEQEVETILDKAMVLFRRFMQEKDVFERV	431
	Cul2	371	ALTSVVNYRE-----PKSV-----CKAPELLAKYCDNLLKKS--AKGMTENEVEDRLTSFTVFKYIDDKDVFFQKF	434
	Cul1	398	ACGFINNNA-VTKMAQSS-----SKSPPELLARYCDSL LKKS--SKNPEAELEDTLNQVMVVFYIEDKDVFFQKF	465
** *				
<i>D.melanogaster</i>	Cul1	465	YSMLAKRLVNHTSASDDAEAMMISK LKQT--CGYEYTVKLQRMFQDIGVSKDLSNYFKQYLAEK-NLTME--IDFGIEVLSSG	543
<i>C.elegans</i>	Cul1	470	YTFMFSKRLLISELSASDEAEANFITKLKSM--CGYEYARLSKMVNDTQVSKDLTADFKKKADM-LGQKS--VEFNVLVSSG	548
<i>S.pombe</i>	Cul1	461	YTKLLAKRLVNGTNSODAESSM LSKLKEV--CGFEYTSKLQRMFQDISLSQEITAFWQLPQSR---AGN--IDFSALVLGTS	537
<i>S.cerevisiae</i>	Cul1	481	YRLLFAKRLIHGTSTSAEDEENIIQLQAA--NSMEYTGKITMFMQDIRLSKILEDDFAVALKNEPDYSKAKYPDLQPFVLAEN	562
<i>H.sapien</i>	Cul5	454	HKAHLTRRLILDISADSEIEENMVLEVRVCPADYVYNKLARMFQDIKVSDELNQAFKEMHKNN-KLALP-ADSVNKKILNAG	534
	Cul4B	607	YKDLAKRLVVGKSASVDAEKSMLSKLKE--CGAFTSKLEGFMKDMELSKDIMIQFKQYMNQ-NVPCN--IELTVNLTMO	685
	Cul3	432	YKQHLARRLLTNKSVSDSEKNMISK LKTE--CGCQFTSKLEGFMFRDMSISNTTMDFFRQHLQAT-QVSLG-GVDLTVRVLTG	511
	Cul2	435	YAMMLAKRLIHGLSMMDSEAMINKLQA--CGYEYTSKLHRMYTDMSVSADLNNKFNFFIKNQ-DTVIDLGISFQIYVLQAG	515
	Cul1	466	YAMMLAKRLVHQNSASDDAEAMMISK LKQA--CGFEYTSKLQRMFQDIGVSKDLSNEQFKHLTNS-E-LD--LDFSILVSSG	543
<i>H.sapien</i>				
<i>D.melanogaster</i>	Cul1	544	SWPFQ--LSN-NFLPSELERSVROFNEFYAARHSGRKLNLWLYQMCKGELIMNVNRNNSST-----YTL	604
<i>C.elegans</i>	Cul1	549	SWPTF--PTT-PITLPQQLSKLIEIFGQFYNEKFNRRRLTWVYSQSRGEITSTAFFKK-----YVF	606
<i>S.pombe</i>	Cul1	538	FWPLS--PNNVNFHLPPELVPLYEGFQNYYSCHNGRKLWLFHLSKGGEIKARINPQTNVT-----YVF	599
<i>S.cerevisiae</i>	Cul1	563	MWPFSS--QVEVEFKLPKELVPSHEKLKESYSQKHNGRI LKWLWPLCRGELKADIGKPGRMP-----FNF	624
<i>H.sapien</i>	Cul5	535	AWSRSS--SEKVFVSLPTELEDLPEVEEFYKKNHSGRKLHWHHLMSNGIITFKNEVGQ-----YDL	593
	Cul4B	686	YWPTY--VPM-EVHLPPPMVKLOEIKFTFYLGKHSGRKLQWQSTLGHCVLKAEFKEGK-----KEL	743
	Cul3	512	YWPTQ--SATPKCNIPAPRHAFEIFRRFYLAHSGRQLTLQHHMGSADLNATFYGPVKKEDGSEVGVCGAQVTGSNTRKHIL	592
	Cul2	516	AWPLTQARSS-TFAIPQLEKSVOMFELFYSQHFSGRKLTLWHYLCTGEVKMNYLGP-----YVA	575
	Cul1	544	SWPFQ--QSC-TFALPSELERSYQRTAFYASRHSGRKLTLWLYQLSKGELVTCFKNR-----YTL	601
<i>H.sapien</i>				
<i>D.melanogaster</i>	Cul1	605	QASTFQMSVLLQFND--QLSFTVQQLQDNTQTQENLIQVLQIL LKA-----VLTSSD-----NENSLTPESTVELFLDYKN	675
<i>C.elegans</i>	Cul1	607	TATTACMCTMLLFNE--QDSYTYEQIAAATKMDEKSAIPAIVGSLIKNL-----VLKADTELQ--KEDEVPMATVSLNKAYMN	680
<i>S.pombe</i>	Cul1	600	QVSTYQMGVLLLYNH--RDSYTYEELAKITGLSTDFTGLNIFLKA-----VLLLDG-----NDKLQDPNSTYKINENFRM	670
<i>S.cerevisiae</i>	Cul1	625	VTTLFQMAILLLYND--ADVLTLNIQEGLSLTIQIHAAAMVPFIKF-----LIQQVPPG--LDALVKPETQFKLSRPFKA	697
<i>H.sapien</i>	Cul5	594	EVTTFLQAVLFAWNQRPREKISFENLKLATELPDAELRRTLWSLVAFPKLRQVLLYEPQVN--SPKDFTEGTLFSVNGEFL	674
	Cul4B	744	QVSLFQTLVLLMFNE--GEESFLEEIKQATGIEDGELRRLQSLACGA--R-VLAKNP-----KGGKIDEDGDKFICNDDFKH	816
	Cul3	593	QVSTFQMTILMLFNN--REKYTFEEIQQETDIPERELVRALQSLACGPTQR-VLTKEP-----KSKEIENGHIFTVNDQFTS	667
	Cul2	576	MVTTYQMAVLLAFNN--SETVSYKELQDSTQMNKEKLTIKSLLDVK-----MINHDS-----EKEDIDAESFSLNMNFSS	646
	Cul1	602	QASTFQMAILLYQNT--EDAYIVQQLTDSTQIKMDI LAQVLQIL LKS-----LLVLEDENANVDEVELKPDILIKLYLYGYN	677
<i>H.sapien</i>				
<i>D.melanogaster</i>	Cul1	676	KK-----RRININQ-PLKT-ELKV-----EQETVHKHIEEDRKLLIQAIVRIMKMRKRLNLTNLISE	731
<i>C.elegans</i>	Cul1	681	KK-----VRVDSKFTMKQ-DAVR-----DTENVQKNVEEDRKSVISACIVRIMKTRKRVQHQQLMTE	737
<i>S.pombe</i>	Cul1	671	KK-----IRVQLNL-PIRS-EQKQ-----ESLETHKTIIEEDRKLLQSAIVRIMKARRTLKHVVLVKE	726
<i>S.cerevisiae</i>	Cul1	698	LK-----TNINFAS-GVKN-DILQSLSCGGCHDNHCKNLGNKRLTEDERIEKELNTERQIFLEACIVRIMKAKRNLPHHTLVNE	773
<i>H.sapien</i>	Cul5	675	INAKVQKRGKINLIGRLQLTTER-----MREENEGLVQLILRLTQEAIIQIMKMRKISNAQLQTE	737
	Cul4B	817	KL-----FRIKINQIMK--ETVE-----EQASTTERVFQDRQYQIDAAIVRIMKMRKTLNLLVSE	872
	Cul3	668	KL-----HRVKIQTVAAKQGESDP-----ERKETRQKVDDDRKHEIAAIVRIMKSRKKMQHNVLAIE	725
	Cul2	647	KR-----TKFKITT-SMQK-DTPQ-----EMEQTSAVEDDRIMYLQAAIVRIMKARKVLRHNAIQE	702
	Cul1	678	KK-----LRVNINV-PMKT-EQKQ-----EQETHKNIEEDRKLLIQAIVRIMKMRKVLKHQQLLGE	733
** *				
<i>D.melanogaster</i>	Cul1	732	VLNQLSTRFKPKVPVVIKKCIDILIEKEYLERMECHKDTYSYLA	774
<i>C.elegans</i>	Cul1	738	VITQLSCRFPKPKVEMIKRCISLIEKEYMLRTEGQKDLLEYLA	780
<i>S.pombe</i>	Cul1	727	TIDQIKSRFTPKVSDIKQCIDMLIEKEYLERQ--GRDEYIYLA	767
<i>S.cerevisiae</i>	Cul1	774	CIAQSHQBFNAKVMVKRAIDSLIQKYLQDGDGEGYAYLA	815
<i>H.sapien</i>	Cul5	738	LVEILKNMFLPQKKMIKEQIEWLIEHKYIRDESINTFIYMA	780
	Cul4B	873	VYNQL--KFPVKPADLKKRIESLIDRYMERDKNPNQYNYIA	913
	Cul3	726	VITQLKARFLSPVVIKKRIEGLIEREYLARTPEDRKVITYVA	768
	Cul2	703	VISQSRARFNPISIMIKKICIEVLIDQYIERSQASADEYSYVA	745
	Cul1	734	VITQLSSRFKPRVPVVIKKCIDILIEKEYLERVDGEKDTYSYLA	776

Figure S7

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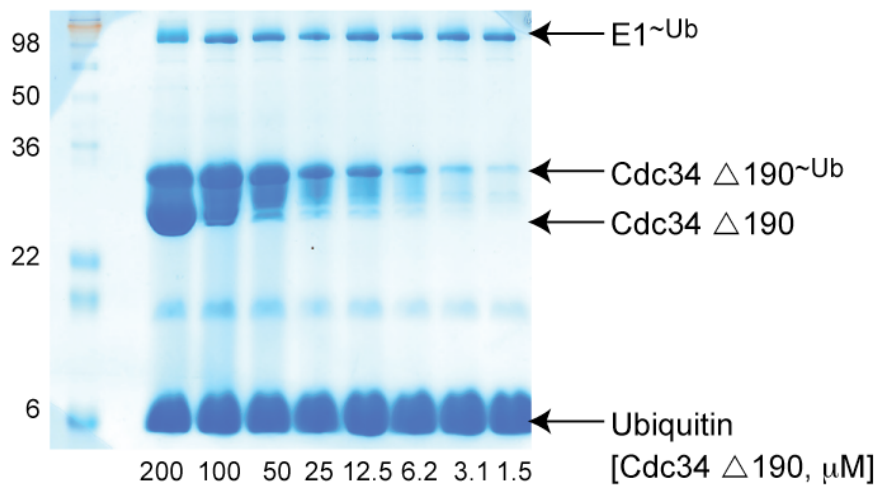


Figure S8

